Datasheet for ABIN5067626

OxiSelect™ Monoamine Oxidase Assay Kit (Fluorometric)

2 Images

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Overview

Quantity:	96 tests
Reactivity:	Others
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The OxiSelect™ Monoamine Oxidase Assay Kit is a simple and sensitive quantitative fluorometric assay for measuring amine oxidase activity in biological samples.
Brand:	OxiSelect™
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Sensitivity:	0.01 U/L
Characteristics:	OxiSelect™ Monoamine Oxidase Assay Kit is a simple HTS-compatible assay for measuring amine oxidase activity. The assay can detect both MAO activity and semicarbizide- sensitive amine oxidases (SSAO). In order to discriminate between MAO-A and MAO-B, the MAO-A inhibitor Clorgyline and MAO-B inhibitor Pargyline are included. In addition, two substrates, Tyramine, and Benzylamine Hydrochloride, are included. Tyramine will react with MAO-A, MAO-B, and SSAO, while Benzylamine will react with MAO-B and SSAO. The two amine oxidase substrates are interchangeable in the assay, and coupled with the provided MAO inhibitors, can be used to determine MAO activity. Applications for the kit include measuring amine oxidase in tissues, blood samples, and screening for amine oxidase inhibitors or substrates. The kit has a detection sensitivity limit of 0.01 U/L. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Product Details

Components:	1. 96-well Microtiter Plate : One 96-well clear bottom black plate 2. 200X Fluorometric Probe : One 55 µL vial 3. HRP : One 100 µL tube of 100 U/mL solution in glycerol 4. Hydrogen Peroxide : One 100 µL amber tube of an 8.82 M solution 5. 100X Tyramine : One 100 µL amber tube (substrate for MAO-A, MAO-B, and SSAO) 6. 100X Benzylamine : One 100 µL amber tube (substrate for MAO-B and SSAO) 7. MAO-A Inhibitor : One 50 µL amber tube of 20 mM Clorgyline solution 8. MAO-B Inhibitor : One 50 µL amber tube of 20 mM Pargyline solution 9. 10X Assay Buffer : One 25 mL bottle

Target Details

Background:	Monoamine Oxidases (MAO) are a collection of flavin adenine dinucleotide oxidoreductase enzymes found in the outer mitochondrial membrane. MAOs catalyze the oxidative deamination of a variety of biogenic and xenobiotic amines. MAOs include MAO-A and MAO-B, which are two isoforms of the enzyme in mammals that have been distinguished based on localization, inhibitor, and substrate specificity. Both MAO-A and MAO-B are omnipresent throughout brain, liver, and other tissues. MAO-A is predominantly found in the liver, intestine, brain, and placenta, whereas MAO-B is found in the liver, brain, and platelets. MAOs primary function is to regulate neurotransmitters such as dopamine, noradrenaline, or serotonin. Dysfunction of MAO enzymes has been associated with many neurological disorders such as depression, drug abuse, migraines, schizophrenia, Attention Deficit Disorder (ADD), Parkinson's disease, Alzheimer's disease, as well as other disorders.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Detects MAO-A and MAO-B in biological samples • MAO substrates and inhibitors included • Hydrogen peroxide standard included • Available with fluorometric or colorimetric detection
Protocol:	The assay can be utilized for both end point and kinetic measurements of Monoamine Oxidase (MAO) activity, as well as semicarbazide- sensitive amine oxidase (SSAO). Monoamine Oxidase reacts with its substrate and generates hydrogen peroxide (H2O2). In the presence of HRP, the Fluorometric Probe reacts with the H2O2 to produce highly fluorescent Resorufin. The Resorufin product can be easily read by a fluorescence microplate reader with an excitation of 530-560 nm and an emission of 590 nm. Fluorescence values are proportional to the amine oxidase levels within the samples. Unknown samples are determined by comparison with a

hydrogen peroxide standard curve. Clorgyline, a specific inhibitor of MAO-A, and Pargyline, a specific inhibitor of MAO-B, are included in the kit to differentiate between the two enzymes. The assay is simple, sensitive, and adaptable to high throughput testing.

Reagent Preparation:

Note: All reagents must be brought to room temperature prior to use. 3

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- MAO-A Inhibitor: Immediately before use, prepare a 100 µM solution in 1X PBS (e.g. Add 5 µL of the 20 mM inhibitor to 0.995 mL 1X PBS). Vortex thoroughly. Store solutions at -20 °C.
- MAO-B Inhibitor: Immediately before use, prepare a 100 µM solution in 1X PBS (e.g. Add 5 µL of the 20 mM inhibitor to 0.995 mL 1X PBS). Vortex thoroughly. Store solutions at -20 °C.
- Assay Working Solution: Immediately before use, prepare an Assay Working Solution using Table 1 below as a guide based on the number of assays needed. Prepare by diluting the 200X Fluorometric Probe 1:100, 100X Tyramine or 100X Benzylamine 1:50, and 100 U/mL HRP to a final concentration of 0.2 U/mL in 1X Assay Buffer. The Assay Working Solution should be protected from light and used within 2 hours. Prepare only enough for immediate use. Note: The Assay Working Solution will appear slightly pink in color. This is normal background and should be subtracted from all fluorescence values. 1X Assay 100X Tyramine HRP 200X Total Volume Number of Assays Buffer (mL) or 100X (µL) Fluorometric of Assay in 96-well Plate Benzylamine Probe (µL) Working (50 µL/well) (µL) Solution (mL) 4.840 100 10 50 5 100 2.420 50 5 25 2.5 50 0.968 20 2 10 1 20 Table 1. Preparation of Assay Working Solution.

Sample Preparation:

Note: Samples should be assayed immediately or stored at -80 °C prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Platelets: Prepare freshly drawn blood in polypropylene or polyethylene tubes with 1/10 volume of 1.5 % EDTA/0.7 % NaCl (e.g. 20 mL blood, 2 mL buffer). The volume of blood depends on the experimental needs. Centrifuge the tubes at 200 x g for 15 minutes at 4 °C. Carefully transfer the supernatant containing platelet rich plasma into clean tubes. Avoid carry over of erythrocytes. Centrifuge at 2000 x g for 10 minutes at 4 °C. Store the platelet pellet at -80 °C until use. Immediately before testing, prepare cell lysate by sonication of the pellet in 1X PBS (Youdim, Ref. 4).
- Cell or tissue mitochondrial fractions: Isolate mitochondria using differential centrifugation for cell or tissue samples, or by the method of choice. Mitochondrial samples can be diluted in 1X PBS. Notes: 4
- All samples should be assayed immediately or stored at -80 °C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.

- A serial dilution will be necessary depending on the total H2O2. Extremely high levels of H2O2 (≥ 500 µM final concentration) can lower the fluorescence because excess H2O2 can further oxidize the reaction product, Resorufin, to nonfluorescent product Resazurin.
- Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µ M will oxidize the probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Tatyana et al, Ref. 2).
- Avoid samples containing DTT or β-mercaptoethanol since Resorufin is not stable in the presense of thiols (above 10 µM).
- Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).

Assay Procedure:

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each sample (H2O2 standard, control or sample) into an individual microtiter plate well. 5
3. If assaying with MAO inhibitors, add 5 µL of the 100 µM inhibitor to the appropriate MAO sample wells. Add 5 µL Assay Buffer to the H2O2 standards and samples without inhibitor. Mix the well contents thoroughly by pipetting or on a horizontal shaker and incubate 30 minutes at room temperature to allow the inhibitor to react with the enzyme. Note: The concentration of MAO-A or MAO-B inhibitors may be adjusted by the user.
4. Add 50 µL of Assay Working Solution to each well. Mix the well contents thoroughly and incubate for 45-60 minutes at room temperature protected from light. Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the kinetics of the reactions.
5. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.

Calculation of Results:

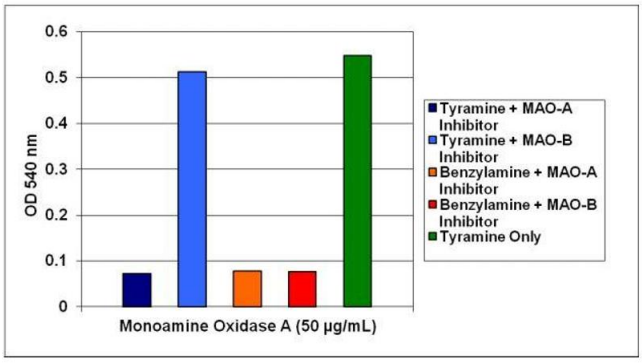
1. Calculate the average fluorescence values for every standard, control, and sample. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected background fluorescence. If sample background control value is high, subtract the sample background control value from the sample reading.
2. Plot the corrected fluorescence for the H2O2 standards against the final concentration of the hydrogen peroxide standards from Table 2 to determine the best slope (µM-1). See Figure 1 for an example standard curve.
3. Use the standard curve to determine the hydrogen peroxide concentration generated by MAO.
4. Determine the monoamine oxidase enzyme activity of the samples using the equation below. Substitute the corrected fluorescence values for each sample. Remember to account for dilution factors. [H2O2] generated MAO Activity (Units/L) = x Sample dilution Reaction time (minutes) Note: One unit of MAO catalyzes the formation of 1 µmole of hydrogen peroxide per minute at 25 °C. 7

Restrictions: For Research Use only

Handling

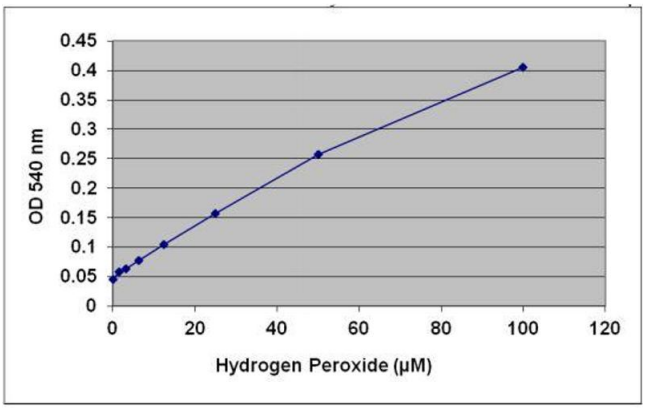
Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, aliquot and store the HRP, 100X Tyramine, 100X Benzylamine, MAO-A Inhibitor, and MAO-B Inhibitor at -20°C. The Fluorometric Probe is light sensitive and must be protected accordingly, it may be stored at either -20°C or -80°C. Avoid multiple freeze/thaw cycles. Store all remaining kit components at 4°C.

Images



Biochemical Assay

Image 1. Measurement of MAO-A. 50 µg/mL of Monoamine Oxidase A was incubated with the MAO-A Inhibitor (Clorgyline) or MAO-B Inhibitor (Pargyline) according to the Assay Protocol. These were subsequently incubated with the substrates Tyramine or Benzylamine within the Assay Working Solution for 45 minutes and read with a microplate reader at 540 nm.



Biochemical Assay

Image 2. H2O2 Standard Curve